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Methods have been developed for removing and manipulating fecal pellets, and techniques for measuring organic carbon in sediment and fecal pellets and lipid content in Diporeia have been learned. A simple and time efficient extraction procedure has been developed to extract 14C-B(a)P and 51Cr from sediment, Diporeia and fecal pellets. In addition, experiments have shown that 51Cr can be used as a conservative tracer for Diporeia and sediment. Good progress has been made in developing the basic methods involved in determining assimilation efficiencies and preliminary data have been collected for the direct measurement and dual-labeled approaches independent of one another.

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ASSIMILATION OF SELECTED PAH AND PCB CONGENERS SORBED TO SEDIMENT BY BENTHIC INVERTEBRATES

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Interim Report for Period 15 Oct 90 TO 14 Oct 91

Prepared for

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Introduction:

Polyaromatic hydrocarbons (PAH's) and polychlorinated biphenyls (PCB's) result from the incomplete combustion of petroleum products and are of concern because they are carcinogenic. Because of their neutral lipophilic nature these chemicals tend to accumulate in sediment. Sediment may act as a sink preventing these compounds from moving back into the food chain. The extent to which the sediment sorption actually hinders reintroduction of these chemicals to the food chain will depend on both the behavior and physiology of organisms residing in the sediment and the physico-chemical interactions between the contaminant and sediment particles. However, assimilation and resuspension of sediment-sorbed contaminants by benthic organisms have been shown to increase exposure of the biota in the food chain to various compounds (Knezovich and Harrison 1988, Landrum 1989). A lack of general assimilation data is evident from the literature, therefore, the process by which invertebrates assimilate sediment-sorbed xenobiotics, requires additional study to better define the role of ingestion in tne process of bioaccumulation.

Two techniques are presently available to determine assimilation efficiencies in invertebrates. However, neither method has been throughly tested. The first method (Lee et al. 1990) measures assimilation efficiencies directly by determining the relative concentrations of ^{14}C-labelled contaminant in the sediment (food) and fecal material. In this method, organisms

are exposed to contaminated sediment and at the end of a test period, fecal pellets and sediment samples are collected. The total organic carbon (TOC) and the ^{14}C-contaminant concentration for the fecal pellets and sediment will be determined and the assimilation efficiency calculated. From this approach, the clam, Macoma nasuta, had an assimilation efficiency for HCB ranging from 39 to 57 % (Lee et al. 1990). An alternative method uses two radioactive isotopes, one of which was assimilated (^{14}C-HCBP) and one that was not ($^{51}Cr^{3+}$) (Klump et al. 1987). Sediment and fecal samples are collected and sampled in a similar manner as described in Lee et al. (1990). However, the assimilation efficiency is calculated from the ratio of the two tracers in the feces and the food. The assimilation efficiency for oligochaetes ingesting hexachlorobiphenyl with sediment ranged from 15 to 36 % and depended on the rate of sediment gut throughput. Even though these assimilation efficiencies from ingested sediments compare with those from ingested food for other chlorinated compounds in fish (Gobas et al. 1988), further research examining the applicability of the above two methods to a variety of organisms as well as under variable environmental conditions is required before the concept of assimilation will be a useful tool in deteriming the fate of chemicals.

Calculating assimilation efficiencies may be complicated by the selective ingestion of size specific sediment particles by invertebrates. For example, oligochaetes preferentially selected the smaller organic rich sediment portion of the bulk sediment

which contained higher concentrations (McMurthy et al. 1983), thus increasing their exposure to the xenobiotics relative to the bulk sediment (Klump et al. 1987). Additionally, the fine grain sediment were the major source of contamination for Great Lakes benthos (Eadie et al. 1985). These findings could lead to substantial errors in the calculations of assimilation efficiencies for contaminants with higher concentrations on organic-rich particles. Landrum (1989) agrees that these problems are important and states that more efforts are required to account for sorption to the fine fraction of sediment and to develop the appropriate normalizing factors.

Chemicals with similar partition coefficients (K_{ow}) should have similar environmental properties (eg. sorption, assimilation, bioavailability, etc.); however, PAH's and chlorinated hydrocarbons of similar lipophilicty had different desorption rates from humic substances when they were held under the same environmental conditions (Landrum et al. 1989). Additionally, chemicals with similar K_{ow}'s values may exhibit variable bioavailability in identical media (Lydy et al. 1989, Landrum et al. 1989). Therefore, the applicability of partition coefficients in predicting assimilation efficiencies of various chemicals will be examined in this study.

The basic objectives of this research are: (1) To measure and compare assimilation efficiencies between two important Great Lakes invertebrate species (Diporeia sp. and Stylodrilius heringianus); (2) To compare the two techniques to determine

assimilation efficiencies and to develop a standard technique that can be used by the Air Force as well as other government agencies; (3) To compare differences between the behavior of 2 PAH (i.e. benzo(a)pyrene and phenanthrene) and 2 PCB (3,3',4,4'-tetrachlorobiphenyl and 2,4,5,2',4',5'-hexachlorobiphenyl) congeners with similar partitioning characteristics.

The first year objectives are: (1) To develop and learn basic techniques needed to perform assimilation experiments with Diporeia in sediment (i.e. indentification and manipulation of fecal pellets and performance of total organic carbon (TOC) and lipid analyses); (2) To develop standard techniques for extracting B(a)P and ^{51}Cr from various media within the aquatic environment (i.e. sediment, Diporeia and fecal pellets); (3) To determine if ^{51}Cr can be used as a conservative tracer for the dual-labeled experiment; (4) To measure assimilation efficiencies of benzo(a)pyrene (B(a)P) in a Diporeia and compare the direct measurement and dual-labeled approaches for determining assimilation efficiencies.

General methodology and procedures:

A majority of the year was spent on working out the logistics for performing assimilation experiments with Diporeia in sediment. A technique was developed and perfected using 50 µl disposable pipets for removing and manipulating the fecal pellets of Diporeia. In addition, techniques for measuring the organic carbon content of sediment and fecal pellet (Gardner 1985) and the lipid content of organisms (Perkin-Elmer 1988) were learned.

Before assimilation efficiencies could be determined, however, techniques had to be developed to extract compounds from sediment. Several techniques were compared and contrasted for extracting B(a)P and ^{51}Cr from sediment. Methods included acid digestion, soxhlet, static brown bottle and sonication techniques. In the acid digestion procedure, wet sediment was placed into a scintillation vial, while perchloric acid and 30% hydrogen peroxide were added as digestive solvents. A one hour digestion period (performed at 60°C) was used, afterwhich scintillation cocktail was added to each vial and the mixture was counted on a scintillation counter. A standard soxhlet procedure utilizing an acetone - cyclohexane solvent mixture (V:V, 1:2) was the second procedure tested. Soxhlets were run for approximately 24 hours, afterwhich, the solvents were concentrated and sampled. The third extraction procedure used was the static brown bottle technique. Samples were dried using anhydrous sodium sulfate and several combinations of solvents were used. A 21 day extraction period was utilized for this technique. The final extraction procedure attempted was the sonication method. The sonication procedure assists the extraction fluid (i.e. scintillation cocktail) in penetrating the sediment matrix; therefore, increasing the extraction efficiency. A Telmar high intensity ultrasonic (375 watt) processor was compared to a Branson low intensity (40 watt) ultrasonic device. The Telmar processor was found to be more consistent, easier to use and considerable more

time efficient. Samples were sonicated for varying amounts of time, using the Telmar processor, in order to determine the amount of time needed for the sonication procedure.

The sonication procedure was also employed for the extraction of ^{14}C B(a)P and ^{51}Cr from Diporeia and fecal pellets obtained from Diporeia. A comparison was made between the extraction ability of cocktail only (no sonication - a generally accepted technique) and cocktail with sonication. The Diporeia were placed into contaminated sediment and allowed to consume sediment for 7 days. Afterwhich, extraction procedures were performed on the Diporeia and fecal pellets.

Another important preliminary test that needed to be conducted was an experiment examining the usefullness of ^{51}Cr as a conservative tracer for the dual-labeled experiment. Diporeia were allowed to feed on ^{51}Cr labelled sediment for 7 days. Subsequently, the Diporeia were removed from the dosing apparatus and placed into clean sediment for 24 h to allow for elimination of contaminated sediment from their guts. A 24 h time frame is sufficiently long to allow for sediment throughput in Diporiea (Quigley, 1988). The Diporeia were then placed into scintillation cocktail, sonicated and counted on a scintillation counter after a two day waiting period. Concentrations were corrected for sorption of ^{51}Cr to the cuticle of Diporeia and these correction values were calculated from a water-only exposure.

Preliminary assimilation experiments have been conducted using the direct measurement and dual-labeled approaches. Methods for the direct measurement approach are presented in Lee et al. (1990), while the methods used in the dual-labeled experiments are found in Klump et al. (1987). Experiments utilizing both direct measurement and dual-labeled approaches simultaneously are currently being conducted.

Status of Results:

In addition to working on fecal pellet manipulations and basic OC and lipid analyses, estimates were also made on uptake clearances (k_s) and sediment sorption coefficients (K_p) for B(a)P sorbed to Florrisant sediment. These kinetic parameters were measured in order to provide a better understanding of the dynamics of this particular sediment system (Tables 1 & 2).

Table 1. Sediment uptake clearance coefficients (k_s) were determined for B(a)P and compared to literature values.
k_s = g dry sediment / g wet organism · h

k_s [1]	k_s [2]
0.0030 ± 0.0003	0.0029 ± 0.0002
3.95×10^{-5}*	1.33×10^{-5}*

[1] Present study.
[2] Landrum and Faust (1991).
* Indicates value normalized for organic carbon of sediment.

Table 2. B(a)P Sediment Sorption Coefficient (K_p & K_{oc})
K_p = ml interstial water / g dry sediment
K_{oc} = ml interstial water / g dry sediment on OC basis

Exposure	K_p	Log K_{oc}
21 day lab[1]	1439 ± 773	5.04
31 day lab[2]	1446 ± 332	5.14

[1] Present study
[2] Landrum and Faust (1991)

Uptake clearance coefficients (k_s) found in this study were consistent with those found for Diporeia in other studies (Table 1) (Landrum and Faust, 1991). However, when the k_s values are normalized for the organic carbon (OC) content of the sediment, the value obtained from the present study is three times as large as that found by Landrum and Faust (1991). The potential reasons for this difference are not known at this time, and this concept should be further tested. The sediment sorption coefficients (K_p) found in the present 21 day study were identical to that found by Landrum and Faust (1991) in a 31 day experiment (Table 2).

All of the methods for extracting ^{14}C labelled B(a)P from sediment worked fairly well. Percent recoveries ranged from 84-90 % (Table 3). However, the sonication procedure was the easiest, quickest and most reproducble of the techniques attempted. Because of the success of the sonication procedure, a decision was made to extract ^{51}Cr labelled sediment only using the sonication and brown bottle methods. The brown bottle

technique was performed for comparison purposes. Percent recoveries were fairly high and consistent for these two methods (Table 3), therefore, the sonication procedure will be used for all of sediment extractions performed in this study. A similar experiment will be performed in the second year of this effort on the remaining PAH and PCB congeners.

Table 3. List of the techniques and results for extraction of ^{14}C labelled B(a)P ^{51}Cr from 1.3% organic carbon content Florrisant sediment.

Procedure	^{14}C-B(a)P % Recovery	^{51}Cr % Recovery	Reproducible	Comments
Acid digestion	84	-------	no	Easy
SOXHLET	90	-------	yes	Time consuming
Brown bottle	85	75	yes	Time consuming
Sonication	84	76	yes	Easy-Best method

The sonication procedure was also successful at extracting ^{14}C-B(a)P and ^{51}Cr from Diporeia and fecal pellets. The Diporeia were vaporized by the sonication process, while the fecal pellets remained fairly intact. The sonication technique appeared to provide a slightly highly DPM value (approximately 10-15% more DPM's detected) for both the Diporeia and fecal pellets in comparison to using the standard cocktail only procedure. Therefore, the sonication procedure will be employed in this study.

Results from the ^{51}Cr conservative tracer experiment indicated that approximately 1% of the ^{51}Cr was found to be associated with the Diporeia in comparison to the dosed sediment; therefore, ^{51}Cr appears to be a good conservative tracer that can be used for the dual-labeled experiments.

Preliminary assimilation experiments have been conducted using the direct measurement and dual-labeled methods. Initial estimates of assimilation efficiencies range from 35-95% for the direct measurement method, while an estimate of 75.6% was determined for the dual-labeled experiment. The large range in assiliation values found in the direct measurement test appear to be the result of a problem encountered in the TOC analysis. For example, the temperature at which the sediments were dried (60 C) was not sufficient to dry all sediments completely; therefore, resulting in a wide range of assimilation values. In future experiments, sediments will be dried at higher temperatures (105°C) hopefully eliminating the variability seen in the assimilation efficiency data. The drying procedure is currently being examined. In addition, experiments utilizing the direct measurement and dual-labeled methods are currently being conducted.

Nine Month Summary:

The majority of the nine month period was spent on working out the logistics for performing assimilation experiments with Diporeia in sediment. Methods have been developed for removing and manipulating fecal pellets, and techniques for measuring OC in sediment and fecal pellets and lipid content in Diporeia have been learned. A simple and time efficient extraction procedure has been developed to extract ^{14}C-B(a)P and ^{51}Cr from sediment, Diporeia and fecal pellets. In addition, experiments have shown that ^{51}Cr can be used as a conservative tracer for Diporeia and sediment. Overall, good progress has been made in the first nine months of this project developing the basic methods involved in determining assimilation efficiencies and preliminary data have been collected for the direct measurement and dual-labeled approaches independent of one another. In the next three months, additional assimilation efficiency values will be determined and method comparisons will be performed.

This work will be presented as a platform presentation at the 12th Annual Society of Environmental Toxicology and Chemistry (SETAC) meeting in Seattle, Washington. Manuscripts resulting from this research will be written and submitted to the Environmental Toxicology and Chemistry Journal.

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Chronological List of Publications Sponsored by the U.S. Air Force Office of Scientific Research - Postdoctoral Award:

Lydy, M.J., J.T. Oris, P.C. Baumann and S.W. Fisher. 1991 (In Press). The Effects of Sediment Organic Carbon Content on the Elimination Rates of Neutral Lipophilic Compounds in the Midge (Chironomus riparius). (Journal of Environmental Toxicology and Chemistry (SETAC)).

Fisher, S.W., M.J. Lydy, J. Barger and P.F. Landrum. 1991 (In Review). Quantitative Strucure Activity Relationships for Predicting the Toxicity of Pesticides in Aquatic Systems with Sediment (Journal of Environmental Toxicology and Chemistry (SETAC)).

Lydy, M.J., W.L. Hayton, A. Staubus, J.T. Oris and S.W. Fisher. 1991 (In Prep.). Pharmacokinetics of Pentachlorophenol and 5,5',6 - Trichlorobiphenyl to Chironomus riparius (Aquatic Toxicology).

Other Contributions:

Landrum, P.F. and M.J. Lydy. 1991. Toxicokinetics Short Course. Society of Environmental Toxicology and Chemistry (SETAC), Seattle, WA., November, 1991.

Chronological List of Presentations:

Lydy, M.J. 1991. The Usefulness of Partition Coefficients in predicting the Fate and Effetcs of Neutral Lipophilic and Moderately Water Soluble Metabolizable Compounds in the Midge, Chironoums riparius. Invited Seminar, The Ohio State University, Columbus, OH. January, 1991.

Lydy, M.J. 1991. Toxicokinetics. Invited Seminar, Eastern Michigan University, Ypsilanti, MI., April, 1991.

Lydy, M.J. 1991. The Usefulness of Partition Coefficients in predicting the Fate and Effetcs of Neutral Lipophilic and Moderately Water Soluble Metabolizable Compounds in the Midge, Chironoums riparius. Invited Seminar, Dow Chemical Company, Midland, MI. May, 1991.

Lydy, M.J. 1991. Quantative Structure Activity Relationships for Predicting the Toxicity of Cholinergic Pesticides in Aquatic Systems with Sediment. Invited Seminar, Exxon Biomedical Inc., East Millstone, NJ. August, 1991.

Lydy, M.J. 1991. The Usefulness of Partition Coefficients in predicting the Fate and Effetcs of Neutral Lipophilic and Moderately Water Soluble Metabolizable Compounds in the Midge, Chironoums riparius. Invited Seminar, Environmental Science Division, Oak Ridge National Lab, Oak Ridge, TN., August, 1991.

Lydy, M.J. and P.F. Landrum. 1991. Assimilation Efficiencies for Selected PAH and PCB Congeners Sorbed to Sediment by Diporeia sp. Society of Environmental Toxicology and Chemistry (SETAC), Seattle, WA., November, 1991.

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Professional Personnel Associated with the Research:

(1) Dr. Peter Landrum, Great Lakes Environmental Research Lab = Post-doctoral Advisor.

(2) Dr. Susan Fisher, Dept. Entomolgy, Dr. Paul Baumann, U.S. Fish & Wildlife Service, Drs. William Hayton and Al Staubus, Dept. of Pharmacy, The Ohio State University; Dr. Jim Oris, Dept. of Zoology, Miami University = Coauthors of Manuscripts.

Fiscal Report

(1) Personnel

Total Salary and Fringe Benefits Charged to this Grant (thru 12/1/91) = $ 30,236.09

(2) Expendible Supplies

Total Expendible Supplies Charged to this Grant (thru 8/1/91) = $4421.16

(3) Permanent Supplies

No permanent supplies were charged to this account.

(4) Travel & Other Expenses

Travel and Other Expenses Charged to this Grant (thru 12/1/91) = $1500.00 (approx.).

(5) Indirect Costs

University overhead (8%) (12/1/91) = $2991.00 (approx.).

(6) Total Costs Charged

$39,148.25

Note that the expendible supplies account is calculated thru 8/1/91. As the grant is written, $6900.00 is allotted for this account. A university wide 3.5% salary and fringe benefit increase, which I received as a post-doc at the Ohio State University has decreased the amount of money in my expendible supplies account from $2478.84 to $1226.75. I will only use monies from the expendible supplies account up to but not exceeding the $40,375 limit.

Second Year Objectives:

(1) Develop a technique for estimating carbon assimilation for Diporeia for use in measuring the reduction in carbon value (RC). The RC value will provide better insight into the estimation of assimilation efficiencies when using the direct measurements method for Diporeia. Currently, Cammen's (1980) estimate of a 22% reduction in carbon efficiency for the polycheate worm (Nereis succinea) is the only measurement available.

(2) Determine the distribution of ^{51}Cr and ^{14}C labeled contaminants among the various particle size classes. This concept is of importance because the dual-labeled method for determining assimilation efficiencies depends heavily upon the equal distribtion of contaminants among all of the particle size classes. Previous studies have not made this measurement.

(3) Extend the assimilation efficiency work done to date with Diporeia to other xenobiotics, including anthracene, 3,3',4,4'-tetrachlorobiphenyl and 2,4,5,2',4',5'-hexachlorobiphenyl. Testing additional chemicals will allow comparisons to be made among the behavior of a series of PAH's and PCB's with similiar partitiong characteristics.

(4) The applicability of partition coefficients in predicting assimilation efficiencies of various chemicals will be examined in this study.

(5) Time permitting, assimilation efficiencies will be determined for an additional species (i.e. the Lumbriculide oligochaete, Stylodrilius heringianus). This work would allow for interspecies comparisons.

(6) Manuscripts resulting from this work will be written and submitted to the Environmental Toxicology and Chemistry journal and will be presented at the annual SETAC meeting.